Surface modification of a porous hydroxyapatite to promote bonded polymer coatings

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Porous hydroxyapatite (Hap) blocks were sintered at several temperatures and methyl methacrylate (MMA) grafted onto the surface in a 2-step heterogeneous system as a model example for surface modification. First, sintered porous Hap was modified with 2-methacryloyloxyethylene isocyanate (MOI) monomer in anhydrous dimethyl sulfoxide using di-*n*-butyltin (IV) dilaurate as a catalyst and hydroquinone as an inhibitor. Amount of the introduction of MOI monomer on porous Hap was 1.62 wt % at sintered temperature 800 °C, 0.68 wt % at it of 1000 °C, and 0.59 wt % at it of 1200 °C. Scanning electron microscopy (SEM) showed that porous Hap pore size and shape before and after MOI treatment were unchanged. Second, graft polymerization with MMA through the vinyl bond on porous Hap was conducted using α,α′-azobis isobutyronitrile (AIBN) as an initiator. Amount of Grafted PMMA on the MOI modified porous Hap was 2.84 wt % at sintered temperature of 800 °C, 6.97 wt % at it of 1000 °C, and 6.27 wt % at it of 1200 °C. MOI-modified and PMMA-grafted porous Hap were characterized using Fourier transform infrared (FT-IR) spectroscopy. The compressive strength of sintered porous Hap with grafted PMMA increased about 2.7–6.7 times compared to intact porous Hap. This 2-step surface modification on porous Hap is widely applicable to graft polymerization with vinyl polymer and conjugation with a protein or an oligopeptide, such as growth factor or an adhesion molecule, to improve Hap mechanical properties and functionality.

1. Introduction

Hydroxyapatite (Hap) has been used in medical applications such as bone implant materials [1–3]. Porous natural corals have also been used because the macroporosity of these materials promotes osteoconductivity and resorption *in vivo*. Walsh *et al*. reported synthesizing unique porous Hap with continuous cavities formed by a foaming calcium phosphate preparation [4, 5]. Porous Hap was applicable for graft cartridges in maxillofacial surgery [6] as alveolar ridge augments and as bone defect filler [7]. Mechanical strength, or cell adhesion and tissue migration on porous Hap, may be limited by its crystallinity, or surface composition and morphology [8–12]. The use of Hap in medical implants would greatly increase if surface modification by an organic compound could improve its mechanical strength or functionality of cell adhesion or multiplication.

Composite preparation of organic materials with Hap has involved the use of coupling agents, such as silanes [13–15], zirconyl salts [16], and polyacid [17], and the introduction of a chemical linkage to octacalcium phosphate by coprecipitation [18, 19]. As is well known, organic compounds with isocyanate groups react readily with Hap surface hydroxyl groups [20].

This paper details a novel 2-step surface modification with an organic compound that improves porous Hap mechanical properties and functionality. We chose poly methyl methacrylate (PMMA) as a typical example for porous Hap surface modification. PMMA is a common polymer used as bone cement for fixing total hip prostheses [15, 18, 19, 21] to give suitable mechanical properties to the material. Initially, 2-methacryloyloxyethylene isocyanate (MOI) possessing a vinyl polymerizable double bond and a reactive isocyanate group at both ends of the compound is reacted with a hydroxyl group of Hap to introduce vinyl groups, applicable as initiation points for grafting PMMA onto porous Hap. We then studied reaction kinetics and the effect on porous Hap shape and microstructure.

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2. Materials and methods

2.1. Materials

The following were used without further purification: calcium hydrogen phosphate dihydrate ($CaHPO_4 \cdot 2H_2O$) and calcium bis(dihydrogen phosphate) monohydrate ($Ca(H_2PO_4)_2 \cdot H_2O$, MCPM) (Wako Pure Chemical Industries, Ltd., Osaka, Japan); calcium carbonate ($CaCO_3$) (Kanto Chemical Co., Inc., Tokyo, Japan); and MOI monomer donated by Showa Denko Co. (Tokyo, Japan). The solvent dimethyl sulfoxide dehydrate (DMSO), the catalyst di-*n*-butyltin (IV) dilaurate, the inhibitor hydroquinone, and the solvent *N*,*N*′-dimethyl formamide dehydrate (DMF) were purchased from Wako Pure Chemical Industries, Ltd. Methyl methacrylate (MMA) was purchased from Wako Pure Chemical Industries, Ltd., and distilled in a vacuum. The initiator α,α′-azobis isobutyronitrile (AIBN) was purchased from Wako Pure Chemical Industries, Ltd., and recrystallized from ethanol.

2.2. Measurements

Porous Hap was characterized by X-ray diffraction (XRD) (Philips PW1729 X-ray diffractometer, The Netherlands) with Ni-filtered Cu Kα radiation (40 kV, 50 mA). JCPDS-PDF card 9-432 was used for XRD reference. We used scanning electron microscopy (SEM, Model EDSEM, JEOL, Tokyo, Japan) at 10 kV acceleration voltage to observe tungsten-coated samples of intact porous Hap and its MOI composites. Infrared spectra (Perkin-Elmer FT-IR Spectrometer Spectrum 2000, USA) were recorded from 4000–500 cm^{-1} using KBr discs. The amount of MOI and grafted PMMA on porous Hap was determined using thermogravimetry-differential thermal analysis (TG-DTA Rigaku Thermo plus TG8120, Japan). About 10 mg of samples were heated to 1200 °C at the heating rate of 20 °C/min. Compressive strength (Texture Analyzer Stable Micro Systems™ TA-XT2i, UK) was measured on 10 mm × 20 mm cylindrical blocks (5) of intact porous Hap and PMMA-grafted porous Hap. Titration was used to determine the amount of MOI added to porous Hap samples as described elsewhere [24–26].

2.3. Chemical modification by MOI monomer

Porous Hap was prepared using a modification of that reported by Walsh *et al.* [4, 5]. Briefly, equimolar tetracalcium phosphate monoxide ($Ca_4(PO_4)_2O$, TCPM), $CaCO_3$, and MCPM were thoroughly mixed and 0.01 N HCl aqueous solution was added to 1 ml against 1 mg of mixed powder. The effervescing mixture was then rapidly mixed in a pestle and mortar before being packed into cylindrical molds. The porous cement block was then dried at room temperature for 24 h before soaking in 10^{-5}N NaOH aqueous solution at 37 °C for 3 days followed by air drying. A porous Hap cylindrical block 10 mm × 20 mm was prepared to cut the cylinders and modified with MOI monomer in a heterogeneous system. Porous Hap was dried for 24 h at 120 °C before use. We immersed 20 pieces of dried porous Hap in 49 ml anhydrous DMSO under nitrogen, and added 1.5 ml of MOI monomer and 0.05 g of di-*n*-butyltin (IV) dilaurate to an anhydrous system containing 150 ppm of hydroquinone. The reaction system was kept at 60 °C for 3, 6, 12, and 24 h. Chemically modified porous Hap was successively washed with DMSO and methanol to remove unreacted reagents. MOI-modified samples were dried at room temperature in a vacuum oven for 24 h.

2.4. Graft polymerization with PMMA

PMMA was grafted via vinyl groups onto porous Hap using AIBN as an initiator; 60 mmol of MMA and 1.0 mol % of AIBN were dissolved in 10 ml of anhydrous DMF. Six MOI-modified porous Hap blocks were immersed in the MMA monomer solution in a 40 ml glass bottle. Graft polymerization was achieved in a nitrogen atmosphere at 60 °C for 24 h. PMMA-grafted porous Hap was copiously washed three times with DMF followed by ethanol washing twice to remove ungrafted homopolymer, and the block was then dried under reduced pressure for 24 h.

3. Results and discussion

3.1. Porous hydroxyapatite

XRD profiles of porous Hap cement unsintered and sintered at 800, 1000, and 1200 °C are shown in Fig. 1(a)–(d). Initially, the reaction between MCPM and $CaCO_3$ formed brushite ($CaHPO_4 \cdot 2H_2O$, DCPD) and simultaneously produced CO_2 gas making interconnecting holes in the bulk [5]. After DCPD and TCPM

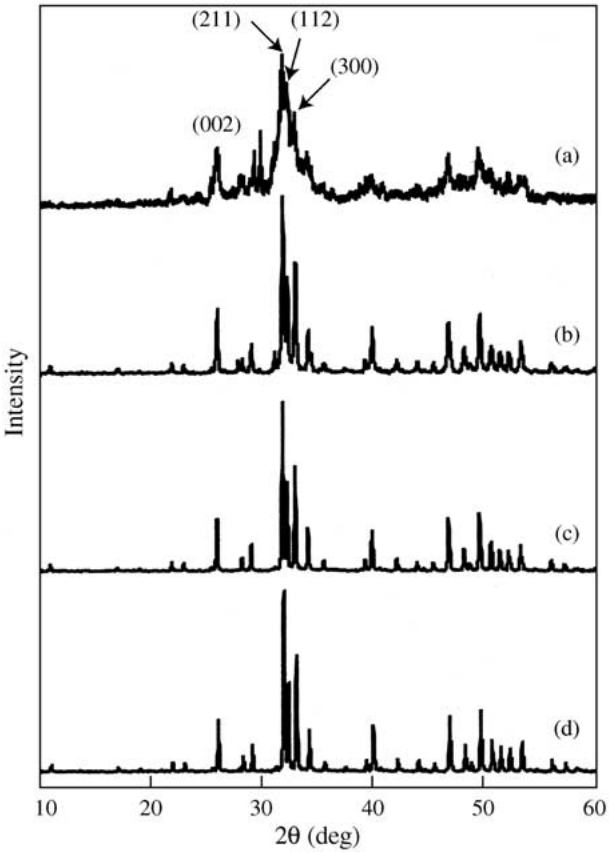

Figure 1 XRD profiles of porous Hap; (a) unsintered; sintered at (b) 800 °C, (c) 1000 °C, and (d) 1200 °C.

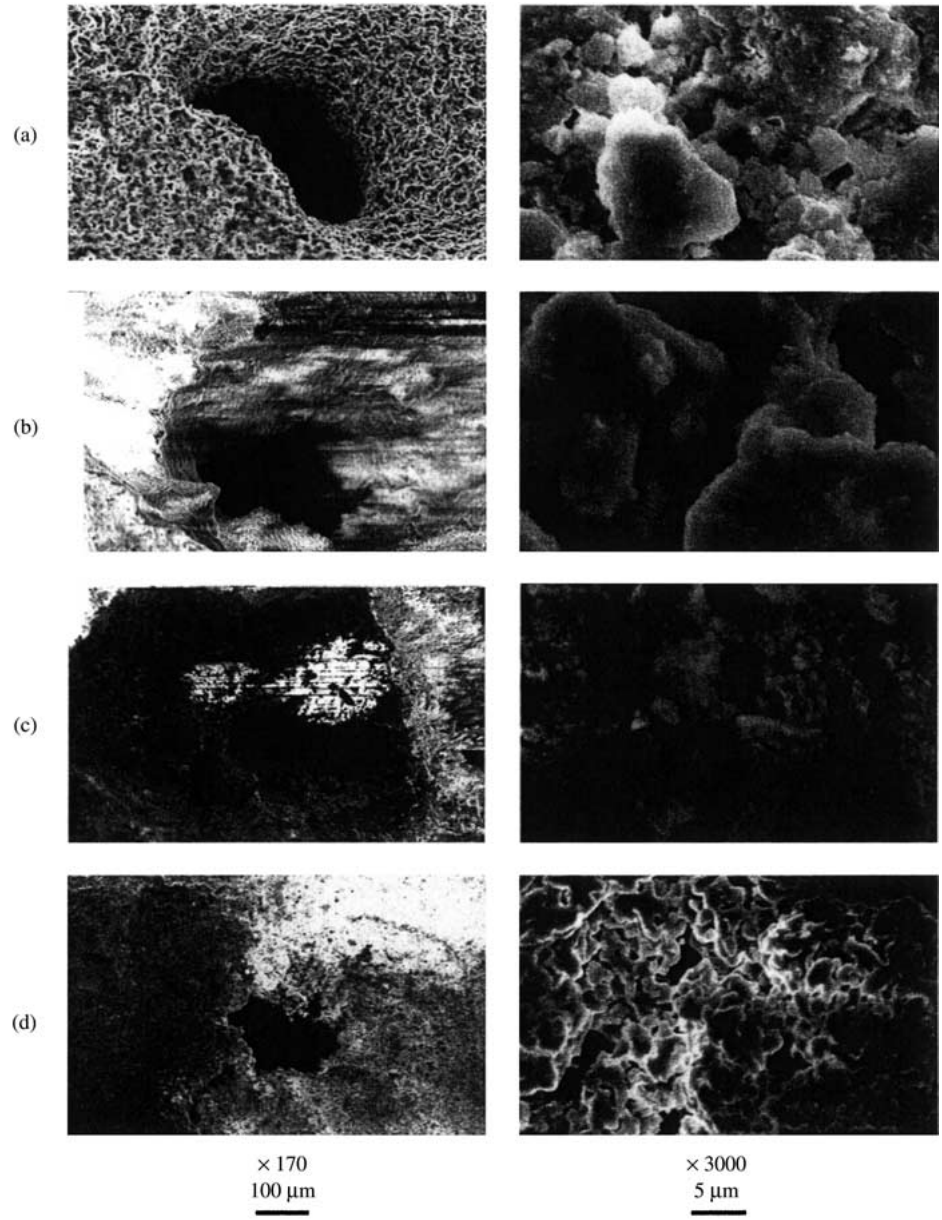

Figure 2 SEM micrographs of porous Hap; (a) unsintered; sintered at (b) 800 °C, (c) 1000 °C, and (d) 1200 °C.

were reacted in the NaOH solution for 3 days, the cement converted to Hap (Fig. 1(a)). With increasing sintering temperature, Hap peaks at (0 0 2), (2 1 1), (1 1 2), and (3 0 0) become sharper due to increasing crystallinity.

Fig. 2 shows a SEM micrograph of samples of macropores and microstructures. The macropore size decreased from 200 to 100 nm with increasing sintering temperature. Hap crystal growth by sintering changed pore size and crystal shape. The microstructure of porous Hap sintered at 800 °C showed a microrugged structure. Samples sintered at 1000 and 1200 °C, however, had a smooth structure. Yubao *et al*. [22] reported that calcium-deficient apatite particles melt about these temperatures, so this change in porous Hap crystal microstructure may be caused by Hap crystal melting during heating from 800 to 1000 °C.

3.2. Introduction of vinyl groups

Porous Hap was chemically modified with MOI monomer in an anhydrous system. Di-*n*-butyltin (IV) dilaurate as a catalyst effectively promoted the reaction between the isocyanate group and hydroxyl groups [23]. We plotted the amount of MOI monomer on porous Hap against reaction time at 60 °C (solid symbols, Fig. 3) determined using TG-DTA to calculate weight loss between room temperature and 600 °C. Open symbols show the amount of MOI monomer on porous Hap calculated by titration to determine the amount of vinyl groups contributing to polymerization [24–26].

It was clear that the polymerization-reaction of MOI by heating during the modification-reaction of the MOI monomer on/in the porous Hap did not occur because the amounts to MOI calculated through the TG-DTA and the titration for 24 h of reaction time took almost same value. MOI monomer added for a 24 h reaction with porous Hap sintered was calculated as 0.11 mmol/g at 800 °C, 0.044 mmol/g at 1000 °C, and 0.038 mmol/g at 1200 °C. In nonsintered Hap powder (BET-specific surface area of 66 m^2/g), Liu *et al*. reported that the amount modified with MOI monomer increased with increasing reaction time, finally reaching equilibrium of about 0.7 mmol/g in both reaction conditions at 50 °C/20 h and 70 °C/12 h [20]. The difference in reaction kinetics between porous

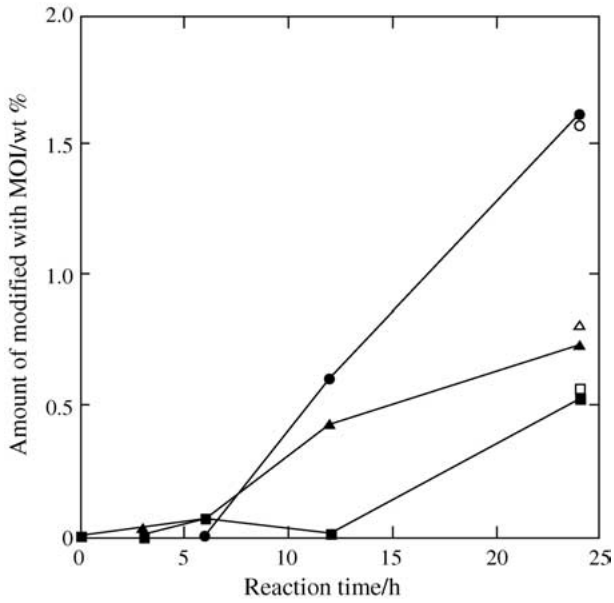

Figure 3 Weight gain of MOI monomer on porous Hap as a function of reaction time; sintered at (●) 800 °C, (▲) 1000 °C, and (■) 1200 °C. Open symbols were determined by titration.

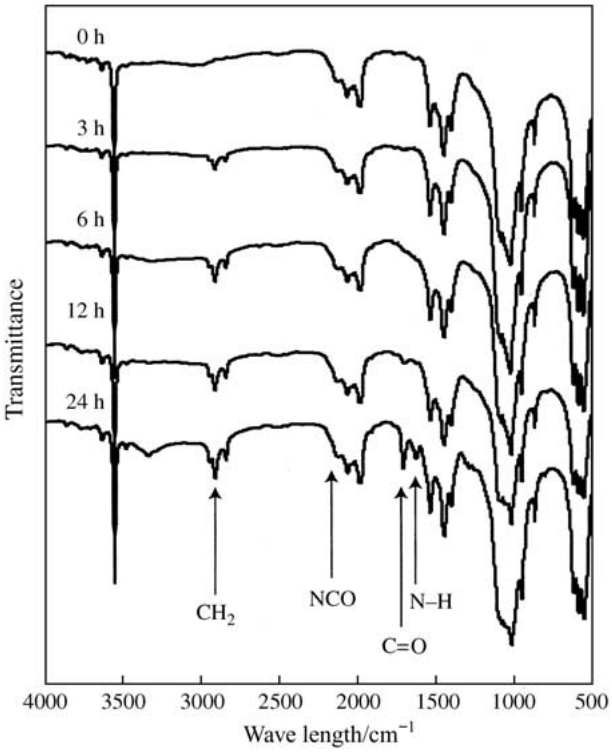

Figure 4 FT-IR spectra of MOI-modified porous Hap sintered at 800 °C. FT-IR spectra of MOI-modified porous Hap sintered at 1000 and 1200 °C were almost identical.

Hap and Hap powder is considered due to the restriction of MOI monomer diffusion to the inside of porous Hap and because the surface area of porous Hap able to react with MOI monomer was much less compared to Hap powder.

In FT-IR spectra of MOI-modified porous Hap sintered at 800 °C (Fig. 4), absorption at 2960 cm^{-1} is attributable to the stretching vibration of C–H, which increased with increasing MOI monomer on porous Hap. The presence of the deformation vibration of the amide N–H peak at 1660 cm^{-1} and of the stretching vibration of the ester C=O peak at 1730 cm^{-1} indicated that MOI monomer and porous Hap were coupled by covalent linkage, the peak attributed to the isocyanate group (–NCO) was clear at 2270 cm^{-1} in the spectrum of the MOI monomer [27]. The isocyanate group peak completely disappeared after MOI monomer was added to porous Hap (Fig. 4), indicating the isocyanate group of the MOI monomer reacted completely with Hap hydroxyl groups under our reaction conditions. The peak of about 1450 cm^{-1} corresponded to ν_3CO_2, the broad band over 1000–1150 cm^{-1} corresponded to ν_3PO_4, the peak of about 630 cm^{-1} corresponded to δOH, and the peaks of about 570 and 600 cm^{-1} to the ν_4PO_4, indicating Hap formation.

Fig. 5 shows SEM observations of MOI-modified porous Hap at low and high magnification. Macro- and microchannels of porous Hap were unchanged by MOI monomer modification compared to Fig. 1, implying that MOI monomer was coated as a thin layer on porous Hap.

3.3. Graft polymerization with MMA

MMA was graft-polymerized on porous Hap through vinyl groups in the MOI monomer using AIBN as a polymerization initiator. We determined the amount of PMMA grafting on MOI-modified porous Hap using TG-DTA (Table I). When graft polymerization with MMA was done using un-modified porous Hap, the amount of graft polymerization of PMMA was zero after washing with DMF. Table I shows that weight gain refers only to the amount of grafting PMMA on porous Hap attached by covalent bonding. MMA grafted on porous Hap sintered at 800 °C was significantly lower compared to that sintered at 1000 and 1200 °C, although MOI modification on porous Hap sintered was significantly higher. There might be two reasons to explain this phenomenon: first, MMA monomer is difficult to diffuse and react the vinyl groups in the micro-cavity of the 800 °C sintered porous Hap, since it has the micro-rugged structure as shown in Fig. 5. Second, sintered porous Hap at 800 °C has too small space that limits the growth reaction of the PMMA graft chain.

FT-IR spectra of PMMA homopolymer and PMMA-grafted porous Hap are shown in Fig. 6. Compared with

TABLE I Weight% of add-on MOI, grafting PMMA, and compressive strength of porous Hap

Sintered temperature/ °C	MOI add-on/ wt%	Grafting polymer (PMMA)/wt%	Compressive strength at intact porous Hap/MPa	Compressive strength at PMMA grafted porous Hap/MPa
800	1.62	2.84	1.54	10.3
1000	0.68	6.97	3.24	21.2
1200	0.59	6.27	17.3	47.1

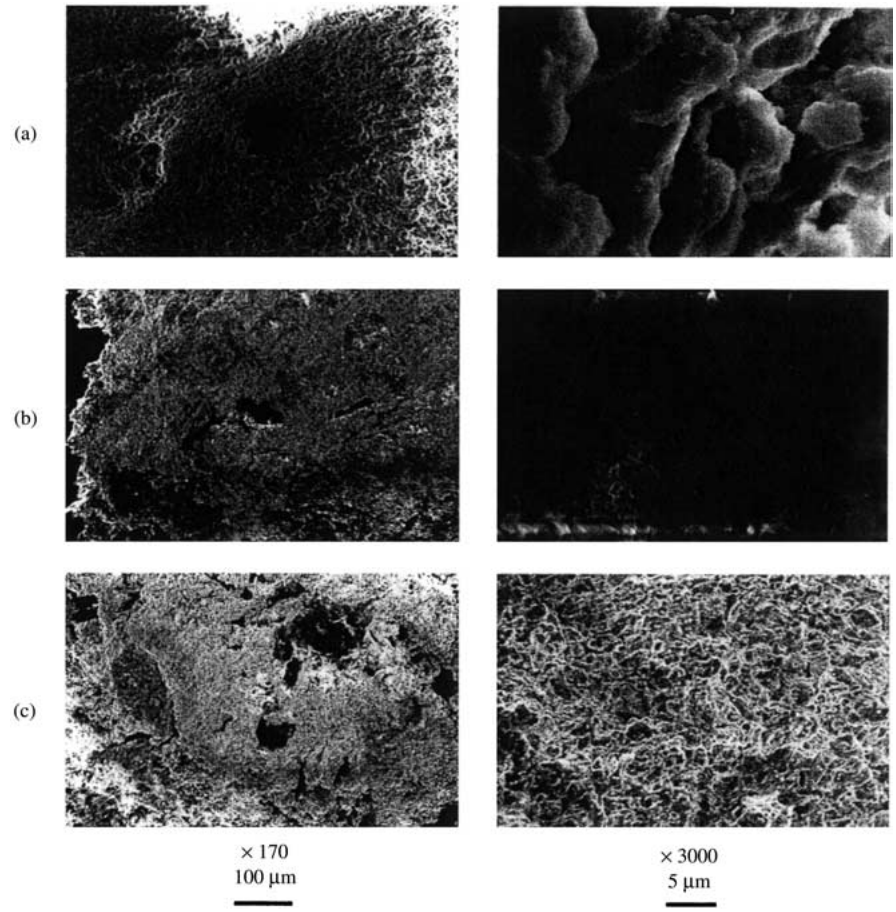

Figure 5 SEM micrographs of MOI-modified porous Hap; sintered at (a) 800 °C, (b) 1000 °C, and (c) 1200 °C.

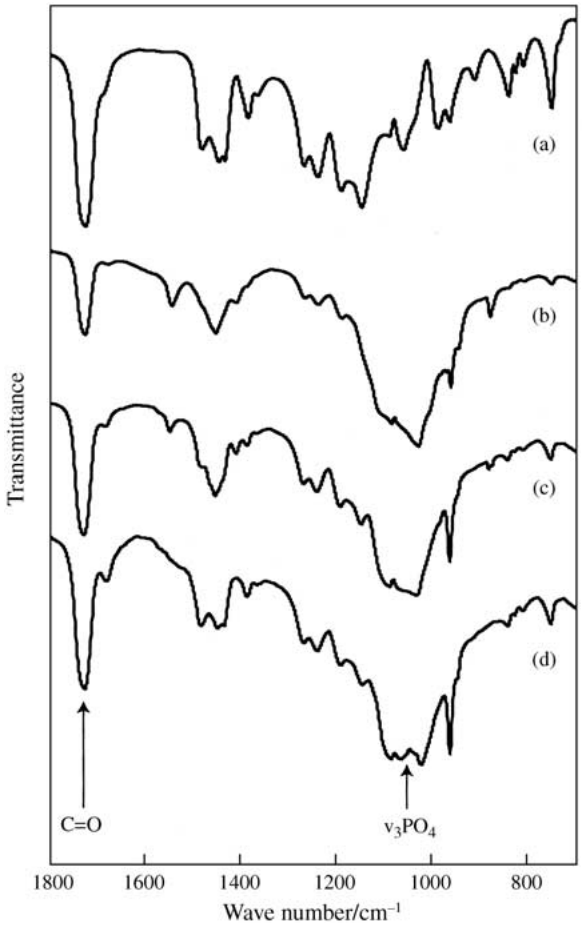

Figure 6 FT-IR spectra of PMMA-grafted porous Hap; (a) PMMA homopolymer; sintered at (b) 800 °C, (c) 1000 °C, and (d) 1200 °C.

Fig. 4, absorption at about 1730 cm^{-1} attributed to the ester C=O peak increased with increasing PMMA grafting, clearly showing that PMMA was grafted successfully on porous Hap. The ratio of the peak strength between the C=O and the PO_4 band of porous Hap sintered at 800 °C (Fig. 6(b)) indicates that the amount of grafted PMMA was less than that of samples sintered at 1000 (c) and 1200 °C (d), which agreed well with PMMA grafted on Hap (Table I).

The compressive strength of intact porous Hap sintered at 800, 1000, and 1200 °C (Table I) increased with increasing sintering temperature, because the crystal growth of porous Hap and apparent density of porous Hap by melting with increasing the sintering temperature. The compressive strength of porous Hap grafted with PMMA increased 4.5 times over the sintering temperature range compared to 11.2 times for uncoated porous Hap. Porous Hap strength thus increased dramatically with this surface modification. For porous Hap sintered at 800 °C, compressive strength increased 6.7 times that of uncoated Hap, even though the amount of PMMA was low. The compressive strength of PMMA-grafted porous Hap sintered at 1000 °C was roughly half that sintered at 1200 °C, although the amount of PMMA grafted on porous Hap sintered at 1000 and 1200 °C was similar. These results indicate that compressive strength could be greatly affected by grafting a thin layer of PMMA on Hap, so we can synthesize porous Hap having desired mechanical properties over a wide range from 1.5 to 47 MPa by controlling the sintering temperature of Hap blocks to be coated and the amount of PMMA then to be grafted.

4. Conclusions

We developed a way to modify the porous Hap surface through an introduced functional group attached by covalent linkage. PMMA provided porous Hap with mechanical and biostable properties through bonding to a vinyl group coupled on porous Hap. PMMA was used as an example polymer for interaction with the Hap surface modified by MOI. This two-step surface modification of Hap is widely applicable to graft polymerization with a vinyl polymer and conjugation with biomolecules, such as proteins or oligopeptides [28], to improve Hap mechanical properties and functionality. We are now developing a 3-dimensional cell-culture vessel and biomolecule separator using porous Hap cartridges grafted with vinyl- and biopolymers.

References

1. I. SOTEN and G. A. OZIN, *J. Mater. Chem.* **9**(3) (1999) 703.
2. L. C. CHOW, *J. Ceram. Soc. Japan.* **99**(10) (1991) 954.
3. M. KIKUCHI, S. ITOH, S. ICHINOSE, K. SHINOMIYA and J. TANAKA, *Biomaterials* **22** (2001) 1705.
4. D. WALSH and S. MANN, in ''Handbook of Biomimetics'', vol. S6, edited by Y. Osada (NTS Inc., Tokyo, Japan, 2000) 59, Chapter 1.
5. D. WALSH, T. FURUZONO and J. TANAKA, *Biomaterials* **22** (2001) 1205.
6. P. YLINEN, M. RAEKALLIO, T. TOIVONEN, K. VIHTONEN and S. VAINONPAA, *J. Oral Maxillofac. Surg.* **49** (1991) 1191.
7. R. W. BUCHOLZ, A. CARLTO and R. HOLMES, *Clin. Orthop.* **240** (1989) 53.
8. T. M. CHU, D. G. ORTON, S. J. HOLLISTER, S. E. FEINBERG and J. W. HALLORAN, *Biomaterials* **23**(5) (2002) 1283.
9. S. JOSCHEK, B. NIES, R. KROTZ and A. GOFERICH, *ibid.* **21**(16) (2000) 1645.
10. W. J. BIGHAM, P. STANLEY, J. M. CAHILL JR, R. W. CURRAN and A. C. PERRY, *Ophthal. Plast. Reconstr. Surg.* **15**(5) (1999) 317.
11. B. K. VAUGHN, A. V. LOMBARDI JR and T. H. MALLORY, *Semin. Arthoplasty.* **2**(4) (1991) 309.
12. G. JIANG and D. SHI, *J. Biomed. Mater. Res.* **43**(1) (1998) 77.
13. A. M. P. DUPRAZ, J. R. DE WIJN, S. A. T. VAN DER MEER and K. DE GROOT, *ibid.* **30** (1996) 231.
14. K. NISHIZAWA, M. TORIYAMA, T. SUZUKI, Y. KAWAMOTO, Y. YOKUGAWA and F. NAGATA, *Chem. Soc. Jpn.* **1** (1995) 63.
15. J. C. BEHIRI, M. BRADEN, S. KHORASANI, D. WIWATTANADATE and W. BONFIELD, in ''Bioceramics'', vol. 4, edited by W. Bonfield, G. W. Hastings and K. E. Tanner (Elsevier Science, London, 1991) 301.
16. D. N. MISRA, *J. Dent. Res.* **12** (1985) 1405.
17. Q. LIU, J. R. DE WIJN, M. VAN TOLEDO, D. BAKKER and C. A. VAN BLITTERSWIJK, *J. Mater. Sci.: Mater. Med.* **7** (1996) 551.
18. V. DELPECH and A. LEBUGLE, *Clin. Mater.* **5** (1990) 209.
19. J. DANDURAND, V. DELPECH, A. LEBUGLE, A. LAMURE and C. LACABANNE, *J. Biomed. Mater. Res.* **24** (1990) 1377.
20. Q. LIU, J. R. DE WIJN and C. A. VAN BLITTERSWIJK, *ibid.* **40**(3) (1998) 490.
21. R. LABELLA, M. BRADEN and S. DEB, *Biomaterials* **15** (1994) 1197.
22. L. YUBAO, C. P. A. T. KLEIN, J. DE WIJN and S. VAN DE MEER, *J. Mater. Sci.: Mater. Med.* **5** (1994) 263.
23. M. R. THOMAS, *J. Coat. Technol.* **55** (1983) 55.
24. A. BAYER, *Ann.* **245** (1888) 103.
25. H. MEYER, *Ber.* **28** (1895) 2965.
26. R. MEYER and E. HARTMANN, *ibid.* **38** (1905) 3956.
27. T. FURUZONO, K. ISHIHARA, N. NAKABAYASHI and Y. TAMADA, *Biomaterials.* **21** (2000) 327.
28. G. T. HERMANSON, ''Bioconjugate Techniques'' (Academic Press, Inc., New York, 1996).

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